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## LIGNIN CHARACTERIZATION - A SUMMARY PROGRESS REPORT

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### ABSTRACT

The objective of this task is the chemical characterization of lignins produced in biomass processing--primarily biomass-to-liquid fuels such as ethanol. This report summarizes selected work carried out in collaboration with several researchers. The areas addressed are: (1) Quantitative structural information on aspen lignins produced in organosolv pretreatment in collaboration with Professor K. V. Sarkanen (University of Washington) and with Dr. D. Robert from the Centre d'Etudes Nucleaires de Grenoble. (2) Structural information on lignins produced by dilute acid hydrolysis of aspen followed by an alkaline delignification in collaboration with Mr. R. Torget, Drs. K. Grohmann and M. Himmel (Biotechnology Res. Branch), and Dr. L. Landucci (U.S. Forest Products Laboratory). (3) Lignin quantitation by nondestructive methods and comparisons of these methods with conventional techniques, in collaboration with Ms. R. Puumala and Dr. Vincent Chiang (Michigan Technological University). (4) The preparation and characterization of lignin model polymers in collaboration with Prof. J. H. P. Utley and Dr. C. Z. Smith (Queen Mary College, University of London) to further the understanding of lignin structure. For instance, the well-defined model compounds prepared by Dr. John Hyatt (Eastman Chemicals Division) up to 1600 molecular weight and the synthetic lignin model polymers prepared at SERI of up to about 10,000 can be used as appropriate calibration standards for the characterization of molecular-weight distributions of lignin polymers in the low-molecular-weight range. Ms. B. Hames (student, Regis College) determined the molar volumes of these lignin model polymers and pertinent low-molecular-weight phenolic compounds. (5) A summary of additional work now in progress is given.

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## INTRODUCTION

The conversion of lignified biomass to ethanol or other fuels involves fractionation of the composite into three principal product streams derived from cellulose, hemicelluloses, and lignin. There are many opportunities to convert the carbohydrate fraction to fuels such as ethanol. Lignin, the structural polymer component of biomass with the highest specific heat of combustion (11,300 BTU/dry lb), is present in 18% to 33% of the weight of the cell wall of wood and in smaller amounts in agricultural residues and herbaceous plants. Lignin utilization as process (solid) fuel is less attractive economically than its sale for upgraded materials, or as a starting feedstock for additional liquid fuels, thus allowing the full utilization of the entire renewable resource in a cost-effective way.

In collaboration with Professor W. Glasser at Virginia Polytechnic Institute and State University, the economic contribution of lignins to ethanol production from biomass was reviewed (Chum et al. 1985a). A first estimate of lignin marginal processing and modification costs and coproduct credits was made. These estimates have been further evaluated and credits from \$0.20 - \$2/gallon of ethanol have been estimated for selected applications from large to relatively small markets, respectively (see Gaines and Karpuk 1987). For instance, the proposed conversion of lignins into a mixture of methyl aryl ethers, fuels fully compatible with gasoline, could lead to about a \$0.35/gallon ethanol credit with an extremely large market.

This task investigates lignin isolation and characterization from biomass-to-ethanol processes. Variables include the pretreatment or fractionation step, the lignin isolation mode, and the starting feedstock. Structural information is important for lignin utilization - as a polymer or as a feedstock for further processing. Lignins from steam explosion, organosolv, and dilute acid hydrolysis are under investigation. Detailed knowledge of lignin structure facilitates decisions on lignin upgrading. In addition, by comparing the various pretreatment lignins, it is possible to suggest methods most amenable for their conversion into liquid fuels.

This report includes brief descriptions of our work. Manuscripts for some of these areas are published or in press (Hawkes et al. 1986, Chum et al. 1987, Chum et al. in press); for others the manuscripts are in preparation. Some of these results have been presented at various national and international conferences (Chum et al. 1987a; Chum et al. 1987b; Johnson et al. 1987; Chum and Johnson 1987). Electrochemical research results were also published as part of a NATO collaborative grant with the Queen Mary College, University of London (Smith et al. 1987 and Ellis et al. in press) and will not be reported here. A short description of the experimental part, results, and discussion has been made on a per-area basis.

# QUANTITATIVE STRUCTURAL STUDIES OF ORGANOSOLV LIGNINS

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Organosolv processes have been reviewed by Sarkanen (1980) (see Organosolv Pretreatment in this Proceedings for additional references). The economics of this type of pretreatment have been compared to that of steam explosion and autooxidation (Chum et al. 1985b) and more recently also with the dilute acid hydrolysis pretreatment (Wright, in press).

A number of organosolv pretreatment experiments with aspen (*Populus tremuloides*) were carried out by Prof. H. Schroeder and Ms. K. Wallace at Colorado State University using a liquor-to-wood ratio of 4:1, which had been shown to be more cost-effective (see Chum et al. 1985b). These experiments followed those initially carried out by Sarkanen and coworkers (1982-1983) using a liquor-to-wood ratio of 10:1. The lignin isolation from the liquors was carried out at SERI. The liquors were evaporated to near dryness in a rotary evaporator at temperatures lower than 60°C under reduced pressure. The solid residue was then leached by redissolution of the hemicellulosic fraction in water, followed by filtration (or centrifugation) of the lignins. Loss of some of the water-soluble lignins occurred. For experiments in the presence of sulfuric and phosphoric acid catalysts comparisons were made between lignin isolation from liquors with and without neutralization prior to evaporation. The pretreatment conditions and yields of isolated lignins and lignin remaining in the cellulosic residues (usually referred to as residual lignin) are given in Table 1. In these experiments, the digester was allowed to cool to room temperature overnight, thus allowing some lignin reprecipitation onto the fibers which explains the relatively high residual lignin contents of some pulps listed in Table 1.

Table 1. Summary of Experimental Conditions Employed in Pulping and Yields of Isolated Lignins. Solvent: Methanol:Water 70:30% by Volume. Cook Time: 2.5 h. Pulping Temperature: 165°C. Liquor-to-Wood Ratio: 4:1.

Cooking Liquor pH	Catalyst	Catalyst Conc., M	Lignin Yields % orig. lignin <sup>a</sup>		
			Water Insoluble	In Pulp	Total
4.3 <sup>b</sup>	None	---			
3.2	NaHSO <sub>4</sub>	0.04	52	39	98
3.0 <sup>c</sup>	H <sub>3</sub> PO <sub>4</sub>	0.05	68	24	96
2.05	H <sub>2</sub> SO <sub>4</sub>	0.05	78	10	88
2.05 <sup>c,d</sup>	H <sub>2</sub> SO <sub>4</sub>	0.05	63	46	109
			89	21	110

<sup>a</sup>These lignins were isolated with the extractives. Klason lignin content of the original wood was 15% and soluble lignins were 3%. Extractives were 3% of the original wood.

<sup>b</sup>Acetic acid added to simulate xylan acetyl group hydrolysis.

<sup>c</sup>Liquors neutralized prior to solvent evaporation.

<sup>d</sup>One hour cook.



Total recovery of lignins in the 88%-98% range was observed for the less acidic pretreatments. The data suggest that 2%-12% of the low-molecular-weight lignins were solubilized in this procedure. In the presence of sulfuric acid at a relatively high concentration, 0.05 M, the apparent recovery of lignins is about 110%, suggesting condensation of carbohydrate-derived materials onto the lignins (possibly to form highly condensed residual lignins).

## EXPERIMENTAL METHODOLOGY

### Spectroscopic Analyses.

**Carbon-13 NMR:** The solvent employed was  $d_6$ -dimethylsulfoxide (DMSO) at 323 K. Lignin solutions ranged 0.5-0.7 g/1.6-1.7 mL. The spectrometers employed were a WM 200 MHz Bruker and a Varian XL-200 at 50.23 MHz. Pulses of 14.5  $\mu$ s for the  $\pi/2$  pulse were employed with pulse delays of 10 sec (unacetylated samples) or 12 sec (acetylated samples) for quantitative spectra. The spectral width employed was 11627 Hz. The number of scans was between 9000-14000 for the quantitative spectra. The Distortionless Enhancement by Polarization Transfer (DEPT) sequence employed was reported by Bardet et al. (1985). The quantitative data are from both the Inverse Gate (I.G.) and DEPT sequences. Major peaks and their assignments (IG spectrum) for a lignin prepared at pH 4.3 are assembled in Table 2. The DEPT subspectra obtained for this sample are shown in Fig. 1.

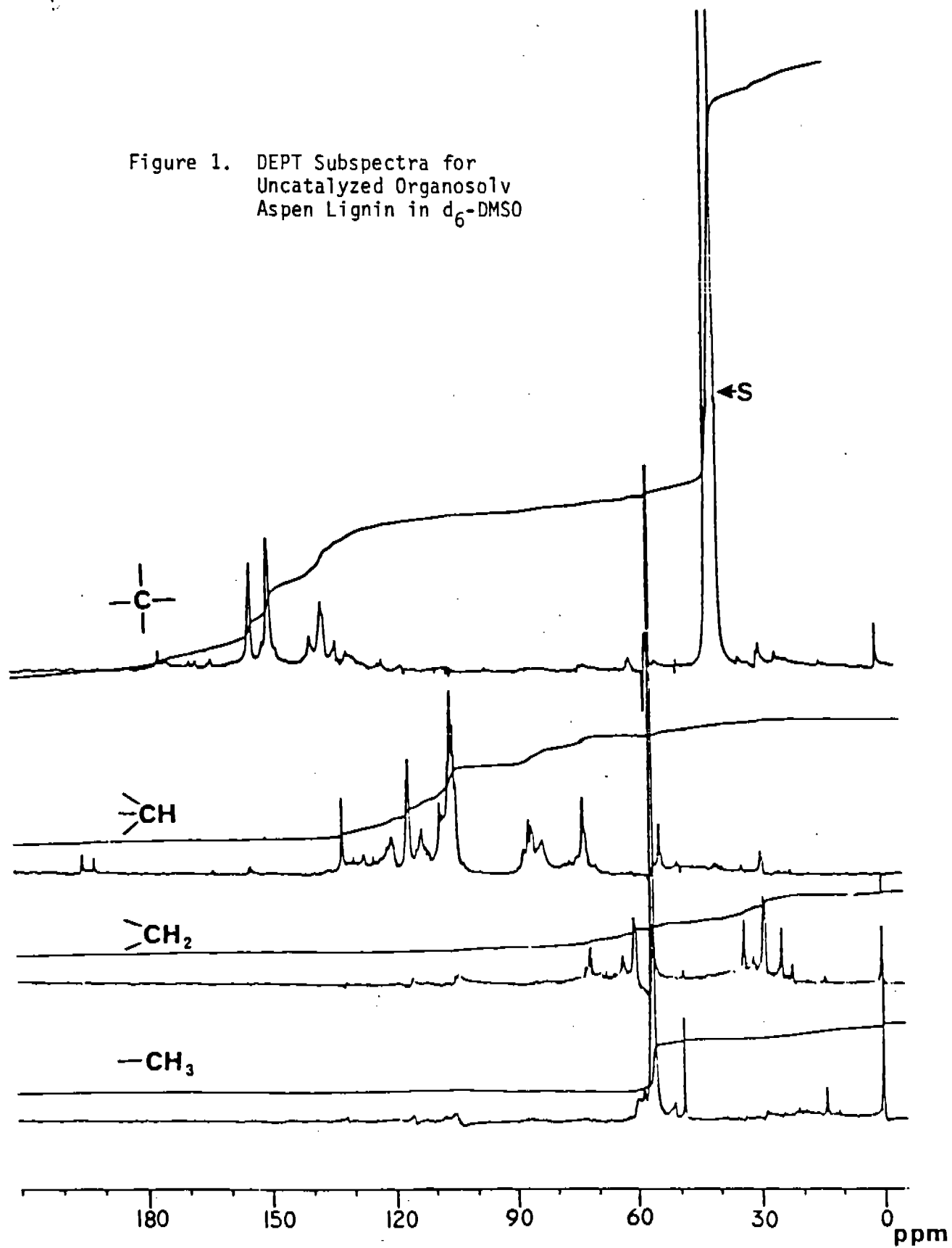
**FTIR Data:** The Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectra were obtained with a Nicolet 5SXC spectrometer fitted with a TGS detector and a spectra-tech center focus DRIFT cell. Each lignin sample was prepared with KBr (Aldrich IR grade 1:100 wt basis). Examples of typical DRIFT spectra of representative lignin samples are shown in Fig 2.

## RESULTS AND DISCUSSION

The composition of selected lignins produced in organosolv pretreatments are assembled in Table 3. The aromatic methoxyl content is given in this table. These preparations have methoxyl groups incorporated in the  $\alpha$ -position of the side chain, which are not detected by the infrared methods employed by Sarkanen et al. (1967) or by Schultz and Glasser (1986) and can be separated from the total methoxyl content in the carbon-13 NMR spectra (see Table 2). The side-chain methoxyl content of these samples adds 0.1 to 0.2 methoxyl groups per  $C_9$  unit, depending on the sample. The total methoxyl group content was determined chemically (Hodges et al. 1979) for a few samples and agreed with the total methoxyl content determined by NMR.

The distribution of hydroxyl groups per aromatic unit in the lignin structures (Robert and Brunow 1984) was calculated from the quantitative carbon-13 NMR spectra of quantitatively acetylated samples (Chum et al. 1985) and is assembled in Table 4. Throughout this section, we will refer to group contents per aromatic unit rather than  $C_9$  unit. The reason for this choice is that as the pretreatment severity increases, the side chain can be severely degraded. There is a marked increase in phenolic hydroxyl content compared to that exhibited by the ball milled aspen lignin. There is also a marked decrease in primary and secondary hydroxyl contents. Some

Figure 1. DEPT Subspectra for  
Uncatalyzed Organosolv  
Aspen Lignin in  $d_6$ -DMSO



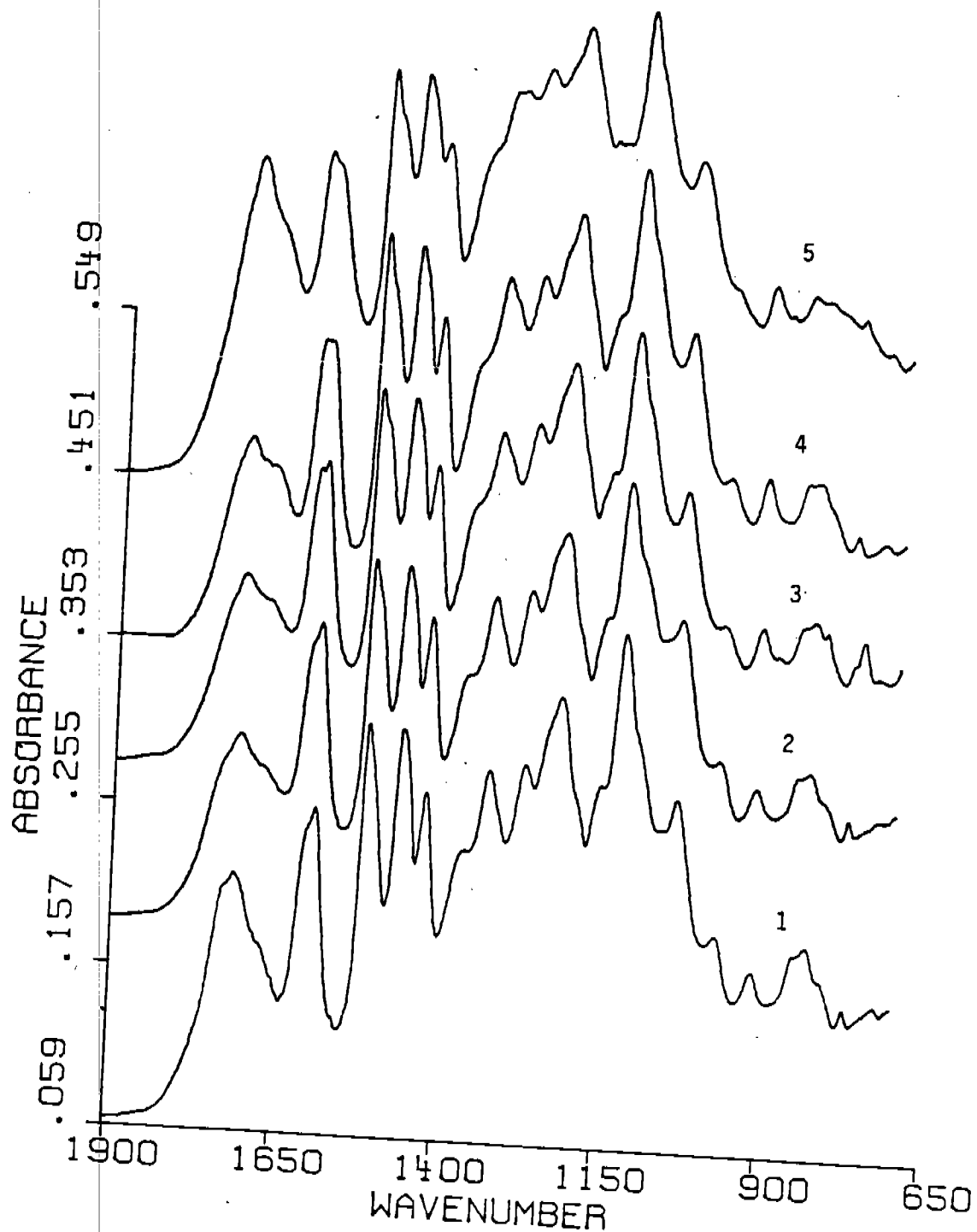


Figure 2. Examples of DRIFT Spectra for Organosolv Lignins. Pulping Conditions of Table 1 and pH of 4.3, 3.2, 2.4, 3.0, and 2.05 for Samples 1, 2, 3, 4, and 5, respectively.

of the decrease in secondary hydroxyl content is balanced by the increase in methoxyl groups in the  $\alpha$ -position. Other characteristic data for side chain and the different types of aromatic carbons are assembled in Table 5. The distribution of the methoxyl groups calculated according to Landucci (1985) is also assembled and the total methoxyl takes into consideration that in these samples p-hydroxybenzoate units are still present. Organosolv processes preserve such units more than steam explosion. The apparent reduction in syringyl groups is probably due to the fact that the hydrolyzed units are in the water-soluble fraction, which is not isolated in the procedure employed.

Table 2. Assignments of Carbon-13 NMR Signals in the Spectra of Aspen Organosolv Lignin Prepared Without Added Catalysts (G = guaiacyl; S = syringyl)

Signal	$\delta$ (ppm)	Assignment
1	194	CHO in cinnamaldehyde
	191.5	CHO in aromatic aldehyde
2	176-172	COOR in aliphatic/aromatic esters
3	170-165	COOR in aromatic/aliphatic esters
4	162	COOR in p-hydroxybenzoates
5	152.3	C-3/C-5 in <u>S</u> $\beta$ -O-4 etherified
	149.4	C-4 in <u>G</u> $\beta$ -O-4 etherified
6	148.1-147.8	C-3 in <u>G</u> $\beta$ -O-4 etherified, C-3 in <u>G</u> non-etherified
8	138.1	C-1/C-4 in <u>S</u> etherified
9	135.2/134.4	C-1 in <u>G</u> etherified, C-4 in $\beta$ - $\beta$
10	131.5	C-2/C-6 in p-hydroxybenzoates
11	129.3	C- $\beta$ vinylic cinnamaldehyde
	121.5	C-1 in p-hydroxybenzoates
12	120.5	C-6 in <u>G</u> etherified and non-etherified
13	115.3-115.5	C-5 in <u>G</u> etherified and non-etherified
14	111.8	C-2 in <u>G</u> etherified and non-etherified
15	104.6	C-2/C-6 in <u>S</u> $\beta$ -O-4 and C( $\alpha$ )HOH
	103.9	C-2/C-6 in <u>S</u>
17	86	C- $\beta$ in <u>S</u> $\beta$ -O-4
	85.5-85.2	C- $\beta$ in <u>G</u> $\beta$ -O-4 and C- $\alpha$ in $\beta$ - $\beta$
18	82.5	C- $\beta$ in $\beta$ -O-4
21	72.5	C- $\alpha$ in $\beta$ -O-4
22	71.4	C- $\gamma$ in syringaresinol
23	60.3-59.9	C- $\gamma$ in $\beta$ -O-4
	58.2	OCH <sub>3</sub> at $\alpha$ -position side chain
24	56.09	OCH <sub>3</sub>
25	53.81	C- $\beta$ in syringaresinol
27	35-32	-CH <sub>2</sub> -
28	28.9-28.5	<u>G</u> -CH <sub>2</sub> - <u>G</u>
29	2-24.5	-CH <sub>2</sub> - aliphatic

Table 3. Analytical Composition of the Various Organosolv Aspen Lignins

Cooking Liquor pH	Elemental % C	Analyses % H	Phen OH %	CO %	C <sub>g</sub> <sup>c</sup> Formula	C <sub>g</sub> Weight	$\bar{M}_n$	Apparent $\bar{M}_w$	$\bar{M}_z$	$\bar{M}_w/\bar{M}_n$
3.2 <sup>a</sup>	62.50	6.30	4.2	1.2	H <sub>8.5</sub> O <sub>2.6</sub> (OCH <sub>3</sub> ) <sub>1.3</sub>	197	610	1750	4910	2.83
3.2 <sup>a</sup>	62.73	6.28	3.9	1.4	H <sub>8.4</sub> O <sub>2.5</sub> (OCH <sub>3</sub> ) <sub>1.3</sub>	197				
3.0	63.75	6.19	4.2	1.6	H <sub>8.2</sub> O <sub>2.4</sub> (OCH <sub>3</sub> ) <sub>1.2</sub>	192	690	1300	3480	1.9
2.4 <sup>a</sup>	64.25	6.20	6.3	1.3	H <sub>8.1</sub> O <sub>2.3</sub> (OCH <sub>3</sub> ) <sub>1.2</sub>	191				
2.4	64.51	6.32	5.4	1.2	H <sub>8.3</sub> O <sub>2.4</sub> (OCH <sub>3</sub> ) <sub>1.2</sub>	190	640	1160	2680	1.8
2.05 <sup>b</sup>	64.78	5.85	4.8	ND	H <sub>7.6</sub> O <sub>2.4</sub> (OCH <sub>3</sub> ) <sub>1.1</sub>	187	560	1140	2010	2.0
2.05	65.67	6.03	4.9	2.8	H <sub>7.7</sub> O <sub>2.1</sub> (OCH <sub>3</sub> ) <sub>1.2</sub>	186	505	940	1510	1.8
MWL	57.65	5.93	1.1	-	H <sub>8.5</sub> O <sub>3.5</sub> (OCH <sub>3</sub> ) <sub>1.4</sub>	217	1500	5100	12060	3.4

<sup>a</sup>Extractives-free wood used to generated these samples.

<sup>b</sup>One hour cooking time.

<sup>c</sup>Methoxyl content per C<sub>g</sub> obtained as average of IR, FTIR, and NMR methods on these samples. Data given correspond to aromatic methoxy contents only.

<sup>d</sup>Molecular weight distribution of acetylated lignin samples determined by high performance size exclusion chromatography as described by Chum et al. 1987.

Table 4. Distribution of Hydroxyl Groups per Aromatic Unit in Extractives-Free Organosolv Lignins Based on Solution NMR of Acetylated Samples

Cooking Liquor pH	Total OH	Phenolic OH	Primary OH	Secondary OH
All Lignins Except for Water-Soluble				
4.3	1.68	0.62	0.66	0.40
3.0	1.10	0.54	0.33	0.22
2.05 <sup>a</sup>	1.00	0.68	0.23	ND
MWL	1.42	0.21	0.75	0.45
Acetone-Soluble Lignins				
3.2 <sup>b</sup>	1.31	0.46	0.50	0.35
2.4 <sup>b</sup>	1.17	0.65	0.37	0.16

<sup>a</sup>One hour cook; <sup>b</sup>Extractives-free wood; lignins isolated were acetone-soluble and represent only a fraction of the total water-insoluble lignins of Table 1.

ND = not detected as secondary hydroxyl.

The major source of the increased phenolic content is the hydrolysis of  $\beta$ -O-4 alkyl aryl ether bonds. Carbon-13 NMR quantitates a fraction of these bonds, namely those which are associated to syringyl units because of the sharp distinct signal at 152.6 ppm due to carbons 3 and 5 in syringyl units that are  $\beta$ -O-4 alkyl ether linked. From this partial measure, it is easily seen that compared to milled wood lignins ( $\beta$ -O-4 ether content/aromatic unit = 0.6), a substantial decrease in this type of ether bonds is seen as the severity of the pretreatment increases (see Table 5). The severity increases with time, temperature, and decreasing liquor pH.

The extractives-free uncatalyzed lignin spectrum (pH 4.3) is the least degraded of the organosolv series and is similar to that exhibited by milled aspen lignin samples, except that because of DEPT's ability to observe the region of 20-40 ppm without solvent effects, it is possible to see that even this sample exhibits condensation signals and a relatively high phenolic hydroxyl content. The three DEPT subspectra for CH<sub>3</sub>, CH<sub>2</sub>, and CH carbons are shown in Fig. 1, as well as the subtraction spectrum from the total quantitative spectra that produces the quaternary carbon spectrum. In addition, through DEPT, p-hydroxybenzoate units can be detected and quantitated (0.08/aromatic unit). A combination of information from DEPT and IG spectra allows one to reconstruct the side chains of these lignins. IG alone provides information on oxygenated side chains (1.9/aromatic unit); the sample also contains minor amounts of cinnamaldehyde-type carbons. DEPT also exposes the distinction between total -CH<sub>2</sub>O- (0.6), terminal -CH<sub>2</sub>OH (0.4) and H-C-O carbons (1.2) for a total of 1.8 carbons/aromatic unit, in excellent agreement with the data from the IG spectrum. A number of -CH<sub>2</sub>- signals can be seen in the 35-32 ppm region and at 28.9 and 28.5 ppm (G-CH<sub>2</sub>-G units linked in the 6-position) for a total of 0.9 carbons/aromatic

Table 5. Distribution of Methoxyl, Aliphatic, and Aromatic Carbons per Aromatic Unit in Organosolv Aspen Lignins Based on Solution NMR of Unacetylated Samples. Numbers in Parentheses are Average Methoxyl Content Determined by FTIR and IR.

Cooking pH	Liquor	Total Methoxyl	Aromatic Methoxyl	Distrib. Methoxyl G%	Syringyl $\beta$ -O-4 Ether S%	Aliphatic O-side Chain
All Lignins Except for Water-Soluble						
4.2		1.46	1.28(1.14)	58	34	0.39
3.0		1.50	1.15(1.3)	71	21	0.21
2.05 <sup>a</sup>		1.55	1.13(1.18)	73	19	0.1
=====						
MWL		1.45	1.45(1.41)	46	46	0.60
=====						
Acetone-Soluble Lignins						
3.2		1.50	1.30(1.21)	56	36	0.46
2.4		1.28	1.20(1.13)	66	26	0.28
=====						
Cooking pH	Liquor	Ternary Carbon	Quaternary Carbon	Condensed Carbon	-CH <sub>2</sub> - 20-40 ppm	
All Lignins Except for Water-Soluble						
4.2		2.2	3.8	0.5	0.9	
3.0		1.9	4.1	1.0	0.5	
2.05 <sup>a</sup>		2.8	3.2	~0	-1.5	
=====						
Acetone-Soluble Lignins						
3.2		2.2	3.8	0.5	0.2	
2.4		1.9	4.1	0.9	0.7	

<sup>a</sup>One hour cook; <sup>b</sup>Distribution between guaiacyl (G) and syringyl (S) units; assumes 8% of p-hydroxy-benzoate units present.

unit. The total side chain identified and quantitated is then:  $1.8 + 0.9 = 2.7$  carbons. Adding the aldehyde, carboxylate, and conjugated unsaturated side chain aldehydes to this number (roughly 0.15/aromatic unit) leads to about 2.9 carbons/aromatic unit, very close to the expected 3 carbons in the side chain. For this lignin, data based on C<sub>9</sub> unit or on aromatic unit should be very close to each other. Included in the side chain structure there is a 0.08/aromatic unit contribution of  $\beta$ - $\beta$  linked structures (syringaresinol structure which have resisted hydrolysis. The integration of the 103-131 ppm region allows the quantitation of the aromatic CH types (ternary carbons) as 2.2, while the integration of the 131-153 ppm allows the quantitation of the quaternary carbons as 3.8. Knowledge of the aromatic CH portion of the spectrum and the aromatic methoxyl content allows one to estimate the amount of condensed carbon units as  $3.8 - (2.2 + 1.3) = 0.5$  (see Table 5).

Similar data were obtained for samples prepared at increasing severity of the pretreatment. Progressively higher degradation of the samples with the increasing severity of treatment is indicated. Very low amounts of xylan that can be detected because of signals at 101.6, 75.5, 73.8, 72.5,

and 65.3 ppm corresponding to C-1, C-4, C-3, C-2, and C-5 in xylan. The sample prepared at pH 3 also exhibited 0.08  $\beta$ - $\beta$  linked units and about 0.04 p-hydroxybenzoate units that resisted hydrolysis. The amount of side chain oxygenated carbons was 1.8 (IG) and  $1.1 (O-C-H) + 0.5 (-CH_2-O) + 0.5 -CH_2-$ . The condensed carbon region was  $4.1 - (1.9 + 1.15) = 1.05/\text{aromatic}$ . Side chain carbons explained were  $1.1 + 0.5 + 0.5 = 2.1$ ; additional carbons from cinnamaldehyde structures and other aldehyde or acid structures could add about 0.15, for a total of roughly 2.3 carbons in side chain/aromatic unit. This lignin is clearly more degraded, as indicated by a lower ether content and a higher degree of condensation.

More severe condition (pH=2.05, for one hour) produced a lignin sample that was considerably more degraded. The oxygenated side chain in this sample has decreased substantially; the signals are also much broader and not as well resolved as the signals for the previous lignin samples. High amounts of  $-CH_2-$  units are present, perhaps as high as 1.5 carbons/aromatic unit. There are still p-hydroxybenzoate units left, perhaps as much as 0.08/aromatic unit. Interesting characteristics of the spectrum are that the amount of aromatic carbons of the CH-type is high (2.8) and the quaternary carbons are relatively low (3.2), which indicate that there is very little condensed carbon structures in the isolated material. It is likely that the more heavily condensed materials are indeed present as residual lignins in the pulp. In this higher severity treatment, it is possible to detect only traces of the syringaresinol units. The low degree of condensation is in agreement with the high side chain carbon content, principally of the  $CH_2$  type suggested by the DEPT spectra. Additional evidence for the types of structures present is provided by the chemical shifts of the acetoxy carbons in the aromatic region ( $-CH_2CH_2CH_2OAc$  or  $-CH_2CH_2OAc$ ). These results of the structures of the acid-degraded lignins are somewhat puzzling in view of the present knowledge of reactions of lignins. It is necessary to assume that in the organosolv medium, additional redox reactions are involved in the production of such  $-CH_2CH_2-$  structures. The spectra are continuing to be analyzed for the possible contribution of furfural-derived structures.

In addition to the NMR analyses, FTIR analysis of these lignins was carried out. It was possible to estimate the hydrolysis fraction for these lignins based on Schultz and Glasser's (1986) proposed correlations between FTIR data and degradative analyses results. The hydrolysis fraction is defined as the ratio of free phenolic hydroxyl groups to total (phenolic hydroxyl and alkyl-aryl ether content) as measured by the corresponding monomeric monobasic benzoic acids produced in the permanganate degradative analyses (see Morohoshi, and Glasser [1979]). As expected based on the NMR data, the hydrolysis fraction increases from 0.2 (milled wood lignin) to 0.8 (uncatalyzed and phosphoric acid catalyzed) to 0.95 (sulfuric acid 1 hour cook) to 1.0 (sulfuric acid 2.5 h). The acetone-soluble lignins present hydrolysis ratios of 0.6 (pH 3.2) and 0.8 (pH 2.4). Thus the FTIR hydrolysis ratio data confirm the NMR trends based on alkyl syringyl ether bonds and extend them to all ether bonds in the lignin polymer. The variation of syringyl ether units accounts for roughly 70% of the total variation at the higher severity pretreatments.



**LIGNINS FROM DILUTE ACID HYDROLYSIS FOLLOWED BY ALKALINE EXTRACTION**  
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Lignins were isolated after dilute acid hydrolysis of aspen by room temperature alkaline delignification or high temperature treatment (140°C) (Grohmann et al.; see this Proceedings). The yields of isolated lignins were 32%, 31%, and 62%, respectively, for room temperature, methanol soxhlet extraction, and high-temperature alkaline extractions. Some analytical data on the lignins produced in these ways are assembled in Table 6. The table also includes comparisons of molecular weight distributions of these lignins. The high temperature alkaline extraction generated the higher polydispersity lignins. The quantitative carbon-13 NMR data on these lignins are assembled in Tables 7 (unacetylated) and 8 (acetylated).

A key difference between these lignins and those from steam explosion or organosolv is the low syringyl content per aromatic unit of the high temperature alkaline extraction than the corresponding samples from organosolv or steam explosion methods, because of the alkaline treatment employed. The samples still preserve approximately 30% of the  $\beta$ -O-4 aryl ether bonds in syringyl units present in milled wood lignin. The spectra of unacetylated lignins have broad peaks in the aromatic region and are similar to those from the more acidic organosolv pretreatment in degree of degradation. Since the samples were not extractives-free, the aliphatic region was only roughly estimated. The oxygenated side chain was quantitated as well as the different types of aromatic carbon. Vinyl groups do not seem to be present in these samples. These lignin samples are similar to those from organosolv and steam explosion methods in general methoxyl, phenolic hydroxyl, ether content, and carbon condensation.

These studies continue with the investigation of a lignin sample prepared by dilute acid hydrolysis of aspen followed by alkaline delignification under milder conditions. The lignins have been prepared and isolated and will, after extraction for removal of extractives, be subjected to the types of analyses described above. Wheat lignin samples from this pretreatment will be investigated.

**METHODS OF LIGNIN QUANTITATION IN LIGNOCELLULOSIC RESIDUES -  
COMPARISON OF DESTRUCTIVE AND NONDESTRUCTIVE METHODS**

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Conventional Methods

Comparisons of classical methods for lignin determination such as Klason lignin (Tappi Method No. T222 os-74) and Kappa number (Tappi Method No. T236 os-76 and Useful Method 246) were performed on lignocellulosic residues from organosolv and dilute acid hydrolysis pretreatments. A linear correlation between Klason and Kappa numbers was observed with a slope of 0.19, an intercept of 0, and an  $R^2 = 0.97$ . The samples that are the most difficult to analyze using the Kappa number method are those from dilute acid hydrolysis (very high Kappa number). These two methods differ greatly in the amount of time for the determination, with the Kappa number

Table 6. Analytical Results on Dilute Acid Hydrolysis Followed by Extraction of Aspen Lignins.

Sample Extraction	Microanalysis			% Phen. OH	% COOH	C <sub>9</sub> Formula	C <sub>9</sub> Weight	Apparent <sup>a</sup>			
	%C	%H	%O					$\bar{M}_n$	$\bar{M}_w$	$\bar{M}_z$	$\bar{M}_w/\bar{M}_n$
25°C/alk	64.09	6.6	28.33	4.2	0.36	H <sub>8.7</sub> O <sub>2.2</sub> (OCH <sub>3</sub> ) <sub>1.3</sub>	194	790	1600	3700	2.0
Methanol	66.53	7.06	26.03	3.0	--	H <sub>9.1</sub> O <sub>1.8</sub> (OCH <sub>3</sub> ) <sub>1.3</sub>	186	660	1300	2900	2.0
140°C/alk	65.20	6.15	28.16	4.2	0.68	H <sub>7.7</sub> O <sub>2.1</sub> (OCH <sub>3</sub> ) <sub>1.3</sub>	189	460	1300	3050	2.8

<sup>a</sup>Determined by high performance size exclusion chromatography in tetrahydrofuran of acetylated lignins.

Table 7. Analytical Results on Dilute Acid Hydrolysis Followed by Extraction of Aspen Lignins Based on Solution NMR of Unacetylated Lignins. All results per aromatic unit.

Extraction Temperature °C	OCH <sub>3</sub>	Methoxy Distribution		b-O-4 Ether Syringyl	Ternary Carbons	Quaternary Carbons	Aliphatic Oxygenated Side Chain	Estimated <sup>a</sup> Aliphatic Side Chain
		Guaiacyl %	Syringyl %					
25/alk	1.25	0.76	0.24	0.25	2.8	3.2	1.4	1.0
140/alk	1.1	0.93	0.07	0.20	2.8	3.2	.9	.3

<sup>a</sup>Rough estimate based on IG spectra; on nonextractives-free lignin.

Table 8. Distribution of Hydroxyl Groups per Aromatic Unit in Dilute Acid Hydrolysis Followed by Extraction of Aspen Lignins Based on Solution NMR of Acetylated Lignins.

Extraction Temperature °C	Total OH	Phenolic OH	Primary OH	Secondary OH
25	1.01	0.53	0.25	0.23
140	1.01	0.58	0.24	0.19

determination itself lasting about 20 min, versus a Klason determination of a few days. Klason lignin determination can be used for the starting material but Kappa number determination only applies to the partially delignified materials. Kappa determinations were performed by R. Puumala while working at SERI on a collaborative project in organosolv delignification in the flow through reactor. Both methods destroy the samples.

### Spectroscopic Methods

A nondestructive method of lignin quantitation based on carbon-13 NMR in the solid state, the cross-polarization magic-angle spinning NMR (CP/MAS C-13 NMR) method was investigated. The method of Haw et al. (1984) was investigated and the method of Hemmingson and Newman (1985) is under analysis. CP/MAS NMR spectra of aspen and wheat straw samples and dilute acid hydrolysis residues and the corresponding residues after sodium hydroxide extraction were obtained by the NMR Regional Center at Colorado State University. The analytical method is based on the aromatic lignin spectral region (105-155 ppm) representing 6 aromatic carbons of the lignin structure; the sum of all carbons in the spectrum representing 100% of the sample carbons, from both carbohydrates and lignin. The calculation of the lignin contents based on these spectra depends most heavily on the C<sub>g</sub> formula assumed for the corresponding lignins.

Using our data from milled wood lignin (see Table 3) and the data from Lee and Pan (1985) for wheat straw, the lignin contents for the starting materials were calculated as 17.4% (aspen) and 16.1% (wheat straw). These numbers compare well with Klason data (18.0% aspen and 19% wheat straw, including about 3% silica). After acid hydrolysis, the lignocellulosic residues were found by NMR to have for aspen 28.7% lignin (29% Klason and equivalent to 30% by Kappa number) and for wheat straw 23% lignin (30% Klason, including silica and ash at about 7%). Room temperature alkaline extraction of the residues led to materials containing 21.6% lignin (aspen) and 8.5% lignin (wheat straw). If the C<sub>g</sub> formulae used are not appropriate, very large errors can result. For instance, if one thoroughly applied a softwood C<sub>g</sub> formula to aspen, the resulting lignin content would be only 14.8%. CP/MAS NMR quantitative data are more accurate for samples with high lignin contents. The datum at 8.5% had at least a 10% error due to the integration accuracy of the aromatic region. The other values had a much smaller integration error ( $\pm 1\%$ ).

A second nondestructive method under investigation is the use of FTIR of the pulps and correlations derived from the literature (or generated from our known samples) to produce quantitative results for the contents of lignin, glucose, and xylose generated upon hydrolysis. Some such correlations have been proposed by Schultz et al. (1985) and found to be unsuitable for other lignocellulosic residues (Grandmaison et al. 1987). We will derive appropriate sets of correlations for aspen hydrolysis residues and test their validity. This method would enable us to completely analyze pulping residues in a very short period of time. In order for the methods to be quantitative and reflect significant physical variation due to the chemical structure of the compounds involved, suitable multivariate analysis using principal component analyses will be employed to select appropriate frequencies and allow better correlations to be developed (see Beebe and



Table 9. Characteristic Polymer Average Molecular Weights from HPSEC and Stereochemical Ratios by NMR

Type:	$\bar{M}_n$	$\bar{M}_w$	Apparent $\bar{M}_z$	$\bar{M}_w/\bar{M}_n$	Erythro/Threo Ratio
EC/TBAP	1540	2270	3020	1.5	
C/TBAH	2600 <sup>a</sup>	3710	4680	1.4	4:1
EC/TEAP	930	1340	1760	1.4	4:1
C/NaOH	3925	7350	1017	1.9	2:1
C/NaOCH <sub>3</sub>	3715	5069	6330	1.4	1:3
C/LiOH	1040	1210	1370	1.2	1:3.7
					1:10

EC = electrochemical preparation; C = chemical preparation; TBAP = tetrabutylammonium perchlorate; TEAP = tetraethylammonium perchlorate; TBAH = tetrabutyl ammonium hydroxide  
<sup>a</sup>Vapor pressure osmometry,  $\bar{M}_n$  = 2550.

In addition to these polymers, substituent effects are under investigation with the collaboration of Dr. Landucci. For instance, by replacing the  $\gamma$ -methyl group with a hydroxymethyl group causes substantial changes to the stereochemistry of the resulting polymer. Addition of sodium hydroxide leads to the formation of a 1:1.5 erythro:threo ratio of isomers versus 1:3 in the case of a model with a  $\gamma$ -methyl group; the polymer formed had an apparent weight-average molecular weight of 1700.

In collaboration with Ms. B. Hames, an undergraduate student from Regis College, the molar volume of these lignin model polymers and related low-molecular-weight compounds is under investigation. Volumes of relevant molecular models were obtained using both CPK molecular models and calculated volumes (Bondi 1964). Linear correlations have been found between the logarithms of the molecular volumes and the retention times obtained from high performance size exclusion chromatography on polystyrene-divinyl benzene copolymer gels.

These studies will provide information on changes in volume, retention times, spectral properties, and fundamental polymer properties in well defined model systems. In addition, the models with well-defined stereochemistry will undergo a conformation analyses using the rotational isomeric state theory and data on time-resolved fluorescence spectra. Such data will allow the obtention of fundamental polymeric property data, which cannot be obtained directly on lignins but can be obtained on these models that will allow the calculation of mean square unperturbed dimensions of such polymers compared to polystyrenes and other commercial polymers. Such studies are presently being carried out on proanthocyanidins, catechin derivatives, by Professor Wayne Mattice and coworkers at the University of Akron (Viswanadhan et al. 1987). These compounds are simple bark components, which are being investigated for their polymeric properties in a number of systems.

These polymers are structurally much closer to lignins than polystyrenes, the standards employed by many authors in the calibration of molecular weight distributions by high performance size exclusion

chromatography. There are problems associated with the use of these standards in high polarity solvents such as DMF and DMF with lithium bromide (see Chum et al. 1987 for a review and results). These lignin polymers and others in preparation, will be very useful for calibrations of molecular weight distributions, one of the most important properties of lignin polymers. Additional polymers will be produced covering a wider range of molecular weights and with more meaningful structures.

#### ADDITIONAL WORK IN PROGRESS

In collaboration with Robert Evans (SERI) and Willem Windig (Geosciences) the development of multivariate analyses on lignins and lignocellulosic residues will continue next year. Such analyses will utilize the data base of well-defined lignins generated by the various pretreatments described above, both of spectral and chemical analyses origin. It is hoped that better basis for correlations will emerge from these studies. Preliminary results are very encouraging. The data base will be expanded to include very well characterized milled hardwood lignin samples that John Obst and Larry Landucci (U.S. Forest Products Laboratory) have prepared. Additional analytical techniques to be investigated will be nucleus exchange analyses (in collaboration with Vincent Chiang and M. Funaoka, Michigan Technological University). In collaboration with ORNL 50 samples of short-rotation species important to the production program will be analyzed jointly with the Forest Products Laboratory (John Obst, V. Schwandt). This analytical data base will be added to the pyrolysis mass spectrometric data on the molecular-beam, mass spectrometer to be obtained by R. Evans and T. Milne in joint work with Professor M. Antal (Hawaii).

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